compound, is capable of emitting a photon, for example a fluorophore or rare earth metal.

[0081] Among such luminescent groups, mention may in particular be made of fluorescein (sodium fluoresceinate) and its derivatives such as fluorescein isothiocyanate (FITC); rhodamine and its derivatives such as tetramethyl rhodamine isothiocyanate (TRITC); diaminidophenyl indo (DAPI); acridine; fluorescent dyes with reactive amines, such as the succinimidyl ester of 6-((7-amino-4-methylcoumarin-3-acetyl)amino)hexanoic acid (AMCA); the fluorescent dyes sold under the brand names Bodipy®, such as Bodipy® FR—Br_2, Bodipy® R6G, Bodipy® TMR, Bodipy® TR and Bodipy® 530/550 (excitation wavelength/emission wavelength, in mm), 558/568, 564/570, 576/589, 581/591, 630/650 and 650/665 sold by the company Bio-Rad Inc. (USA), the dyes Cascade Blue (Trilink BioTechnologies (USA), Cy2, Cy3, Cy3.5, Cy5, Cy5.5 and Cy7 (Bio-Rad Inc., USA), Dabcyl® and Edans® (Eurogentec, BE); eosin; erythrosine; 6-Fam and Texas Red.

[0082] According to the invention, the term "receptor" is intended to mean any substance capable of forming a noncovalent and reversible linkage (complex) with the analyte, an analog of the analyte or a fragment of the latter. This receptor is of course chosen according to the nature of the molecule (B) of the tripod Y.

[0083] These receptors can in particular be chosen from compounds that are biological in nature (antibodies in whole, fragmented or recombinant form (Fab', Fab, scFv), receptors, polynucleic acids (DNA or RNA), peptide nucleic acids, lectins or alternatively transporter proteins) and compounds that are chemical in nature, such as, for example, specific synthetic receptors and chelates.

[0084] Among such receptors, mention may, for example, be made of monoclonal anti-substance P, anti-prion protein or anti-angiotensin II antibodies, polyhistidine, the nitrilotriacetic acid-nickel (NTA-nickel) system, and complementary nucleotide probes.

[0085] According to a preferred variant of the method of detection in accordance with the invention, said receptor exhibits greater affinity for the analyte a than for the molecule (B).

[0086] According to the invention, the term "quenching compound" (Q) is intended to mean any molecule that allows a decrease in or the disappearance of the luminescence of the luminescent compound (L) when the receptor is complexed with the molecule (B). This compound, that may be diverse in nature, may in particular be a chemical compound (luminescent or nonluminescent), a heavy atom or a nanoparticle.

[0087] Among such compounds (Q), mention may in particular be made of fluorescent compounds such as those mentioned above for the groups L, rhodamine and its derivatives such as tetramethyl rhodamine (TMR), nonfluorescent molecules such as the compounds sold under the brand names Black Hole Quencher® 1, 2 and 3 (Biosearch Technologies), Nanogold Particules® (Nanoprobes), Eclipse Dark Quencher® (Epoch Biosciences), Elle Quencher® (Oswell), malachite green, and the dyes QSY® 7, QSY® 9 and QSY® 21 (Molecular Probes).

[0088] When the molecule (B) is a peptide or an oligonucleotide, then the tripod Y used according to the method in accordance with the invention can be prepared by carrying out a peptide or oligonucleotide synthesis during which at least one amino acid (or modified nucleotide) comprising a function (F_1) and another amino acid (or modified nucleotide) comprising a function (F_2) is added to said molecule (B), F_1 and F_2 having the same meanings as those indicated above. In this case, F_1 and F_2 also provide the linkage respectively with the luminescent compound (L) and the surface of the solid phase. Just as above, the amino acids (or nucleotides) comprising the chemical functions F_1 and F_2 can also be replaced with an amino acid (or nucleotide) coupled to a biotin (for example, the product 9-fluorenyl-methoxycarbonyl (Fmoc)-lysine(biotin)-OH, sold by the company Calbiochem-Novabiochem AG). In this case, this amino acid (or nucleotide) will provide the linkage with the surface of the solid phase or with the luminescent compound (L) to which streptavidin (or neutravidin or avidin) will have been coupled beforehand, so as to form an indirect linkage.

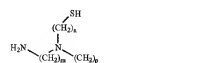
[0089] Among the complexes C of formula (I) in accordance with the invention, mention may in particular be made of the compounds in which:

[0090] i) (B) is chosen from peptides, proteins, oligonucleotides, sugars and peptide nucleic acids,

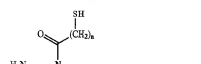
[0091] ii) L is fluorescein, and

[0092] iii) the backbone of the tripod Y is chosen from the structures Y_1 to Y_3 below:

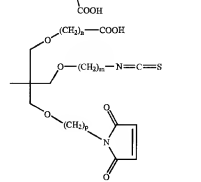
(Y'1)



(Y'2)



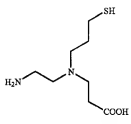
(Y'3)



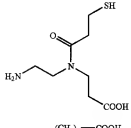
[0093] in which n, m and p, which may be identical or different, are integers between 1 and 20 inclusive.

[0094] Among the structures Y_1 to Y_3, the compounds of formulae (Y'_1) to (Y'_3) below are particularly preferred:

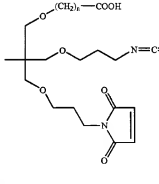
(Y'_1)



(Y'_2)



(Y'_3)



[0095] The tripods Y used in the method in accordance with the invention can be prepared by analogy according to the methods of organic and peptide synthesis conventionally used and well known to those skilled in the art.

[0096] The complexes C of the formula (I) as described above are compounds that are novel in themselves, which, in this respect, constitutes another subject of the invention.

[0097] These complexes C of the formula (I) can be prepared by complexing a tripod Y in accordance with the invention with a receptor-Q, according to the methods conventionally used in the state of the art.

[0098] A subject of the invention is also the use of at least one complex C of formula (I) in a method for continuous heterogeneous-phase detection of an analyte a in a fluid sample.

[0099] Finally, a subject of the invention is a device for continuous heterogeneous-phase detection of at least one analyte a in a fluid sample, said device being characterized in that a fluid sample to be analyzed is integrated into a medium forming a stream that flows over at least one solid support at the surface of which is attached at least one tripod Y in accordance with the invention as described above and specific for the analyte a to be detected, a luminescence detector placed opposite the solid support is coupled to a valve control that is controlled by a threshold of intensity of signal emitted by the detector and which triggers, for a given period of time, the opening of a reservoir containing a receptor-Q capable of forming a complex with the tripod Y, this reservoir being linked to the support via a feedback loop which comes in upstream of the solid support to which the tripod Y is attached, in order to saturate and/or regenerate the latter with receptor-Q by passage in the stream and complexation on the tripod Y.

[0100] According to specific embodiments:

[0101] the luminescence intensity values are monitored and secondarily translated into an amount of analyte a by a calculation system coupled to the luminescence detector;

[0102] an event marker, for instance an alarm, is placed in the feedback loop in order to signal a variation in intensity of the signal above a predetermined value;

[0103] the solid support is a capillary coupled to the environment containing the sample to be analyzed, the coupling being carried out either by means of a round-bottomed capture flask in which the sample sparges in a medium corresponding to that of the flow stream, or by means of a flexible pipe;

[0104] the stream is entrained by means of the low pressure produced by a pump, a piston, or equivalent.

[0105] These devices can in particular be used for detecting the presence of an analyte a in a natural medium, for instance especially in lakes and rivers, or in industrial media such as swimming pools, factories, purification plants, ventilation or air-conditioning systems, etc. Where appropriate, they can be equipped with a round-bottomed capture flask and with a sparging system for collecting samples in gaseous form, such as air, and for solubilizing the constituents to be detected that they contain.

[0106] Besides the above provisions, the invention also comprises other provisions that will emerge from the following description, which makes reference to examples of synthesis of backbones of tripods Y, to an example of synthesis of a complex of formula (I) and to two examples of detection of substance P in a fluid sample, and also to the attached FIGS. **1** to **8** in which:

[0107] **FIG. 1** illustrates a general view of a nonlimiting example of a heterogeneous-phase detection device according to the invention, for measuring the presence of an analyte a in a water purification plant tank. According to this device, a piece of flexible tubing **1** connected to a tank outlet is mounted on a capillary tube **3**. This tube serves as a support for the attachment of two types of tripod according to the invention, namely Ya and Yb, allowing the specific detection of two types of analytes. The liquid sample originating from the tank flows as a continuous stream (arrows Fa) in the capillary **3** by means of the action of a peristaltic pump **5**, and is then evacuated. In the vicinity of the capillary and opposite each area of the capillary to which the tripods Ya and Yb are attached, the placement of a fluorimeter, **7***a* and **7***b*, for detecting fluorescence is envisioned. Each detector is connected to a reservoir of receptors-Q, **9***a* and **9***b*, respectively specific for the tripods Ya and Yb. The reservoirs are connected, via side channels **11***a* and **11***b* and a common channel **11***c*, to the capillary **3**, upstream of the

areas to which the tripods Ya and Yb are attached, relative to the direction of flow of the stream. The common channel is welded perpendicular to the capillary **3** and all the channels form feedback loops. Each detector is coupled to a valve control, respectively **13***a* and **13***b*, for opening the corresponding reservoir, respectively **9***a* and **9***b*, and so as to allow its content to pour out into the capillary **3** (arrows Fb) and then to complex on the corresponding specific tripod, Ya or Yb, via the channels **11***a*, **11***b* and **11***c*, in order to regenerate this tripod. In this example, the regeneration can be carried out continuously, the threshold for triggering this regeneration operation corresponding to the minimum variation in luminescence that can be detected according to the sensitivity of the detector. An event marker **15***a* (respectively **15***b*) is mounted in each feedback loop. In operating mode, and after calibration, the intensity of the fluorescent signal, Ia and Ib, emitted by the tripods Ya and Yb is measured by the fluorimeter, which makes it possible to calculate, using an attached calculating device (not represented), the average concentration of analyte under examination in the sample. The valve opening control **13***a* (respectively **13***b*) is controlled by the intensity of fluorescent signal measured by the corresponding detector. Opening of the corresponding valve is then triggered for the period of time corresponding to the regeneration of the tripods concerned. If the analyte a is present in the sample during the regeneration, this results in an increase in the duration thereof (some of the receptor-Q complexing with the analyte a). The duration of the regeneration can be determined by the time necessary for the fluorescence to return to the basal level; if this is greater than a predetermined value, an event marker may be triggered. When the intensity of the signal varies by an amount greater than a predetermined value, for a given time interval, the event marker **15***a* (respectively **15***b*) signals this fact by means of a visual and/or sound warning **16**. So if the fluorescent signal corresponds to all the analytes a that have passed through the capillary between two regenerations, the fluorescence can also be read at regular time intervals. In other examples, when the sample is a gas, the medium used and the means for creating a stream are adapted by those skilled in the art;

[0108] **FIG. 2** represents the fluorescence measured, in arbitrary units, after immobilization on a microtitration plate, via neutravidin, of a tripod Y comprising fluorescein as compound (L) and a substance P analog as molecule (B), as a function of the concentration of tripod in μM;

[0109] **FIG. 3** represents the curve for decrease in the fluorescence (in arbitrary units) of a tripod comprising fluorescein as compound (L) and a substance P analog as molecule (B), as a function of the amount of tetramethyl rhodamine-labeled anti-substance P monoclonal antibody (mAb SP31-TMR) (in nM) that complexes with the tripod;

[0110] **FIG. 4** represents the percentage inhibition by substance P of the decrease in fluorescence caused by the mAb SP31-TMR (% ID), expressed as a function of the concentration of substance P (in nM);

[0111] **FIG. 5** represents the fluorescence measured (in arbitrary units) after the bringing into contact of a solid support to which a tripod Y in accordance with the invention is attached, said tripod possibly being complexed with the mAb SP31-TMR (well FI: fluorescence measured in the absence of mAb SP31-TMR and of substance P and well Fl_0: fluorescence measured in the presence of mAb SP31-TMR but in the absence of substance P), with various concentrations of substance P (well FL_x: fluorescence measured in the presence of mAb SP31-TMR with x=concentration of substance P: 1; 0.1 or 0.01 μM), during a first assay, and then after regeneration of the support and, finally, after the wells have again been brought into contact with the three concentrations of substance P;

[0112] **FIG. 6** represents the mean fluorescence measured (in arbitrary units) for each of the wells FI, Fl_0 and Fl_x, after 11 substance P-assay and regeneration cycles;

[0113] **FIG. 7** represents the fluorescence (in arbitrary units) measured for each of the 11 assays carried out in wells FI, Fl_0 and Fl_x, with x=1 μM of substance P;

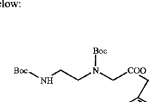
[0114] **FIG. 8** represents the change in fluorescence (in arbitrary units) as a function of time, of a capillary functionalized with a tripod Y in accordance with the invention, brought into contact with a labeled monoclonal antibody mAb SP31 (zone 1), and then with substance P (zone 2), and regenerated by bringing the tripod attached to the capillary into contact with the labeled monoclonal antibody mAb SP31 (zone 3), the last two steps being repeated three times.

EXAMPLE 1

Preparation of a Backbone of Formula (Y'$_1$) of a Tripod Y In Accordance with the Invention

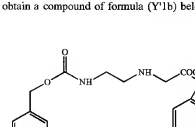
[0115] One equivalent of 1,2-ethyldiamine is reacted with two equivalents of $(Boc)_2O$ to give bis(tert-butyloxycarbonylamino)-1,2-ethyl, which is subsequently reacted with one equivalent of phenyl 3-bromopropanoate in the presence of sodium hydride to give the compound of formula (Y'1a) below:

(Y'1a)



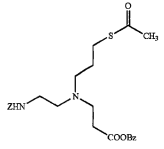
[0116] The compound Y'1a is deprotected in a trifluoroacetic medium and then the deprotected compound obtained is condensed in the presence of triethylamine (TEA) and of one equivalent of Z chloride (with Z=carbobenzyloxy), so as to obtain a compound of formula (Y'1b) below:

(Y'1b)



[0117] The compound of formula (Y'1b) then gives, in the presence of S-(3-chloropropyl)ethanethioate and of TEA, the compound of formula (Y'1c) below:

(Y'1c)



[0118] in which Z has the same meaning as that indicated for the compound of formula (Y'1b) above and Bz denotes a benzyl group.

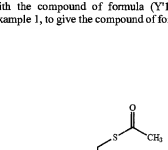
[0119] The acid and primary amine functions of the compound of formula (Y'1c) are deprotected in the presence of palladium-on-charcoal and the thiol function is deprotected by the action of hydroxylamine, to give the compound of formula (Y'1).

EXAMPLE 2

Preparation of a Backbone of Formula (Y'_2) of a Tripod Y in Accordance with the Invention

[0120] 3-[(2-Oxopropyl)thio]propanoic acid, prepared from 3-bromopropanoic acid and from ethanethioic acid, is reacted with the compound of formula (Y'1b) obtained above in example 1, to give the compound of formula (Y'2a) below:

(Y'2a)



[0121] in which Z denotes a carbobenzyloxy group and Bz denotes a benzyl group.

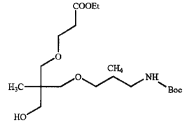
[0122] The acid and primary amine functions of the compound of formula (Y'2a) above are deprotected in the presence of palladium-on-charcoal and the thiol function is deprotected by the action of hydroxylamine, to give the compound of formula (Y'2) described above.

EXAMPLE 3

Preparation of a Backbone of Formula (Y'_3) of a Tripod Y in Accordance with the Invention

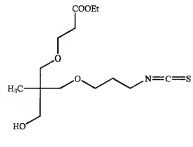
[0123] One equivalent of 2-(hydroxymethyl)-2-methylpropane-1,3-diol and one equivalent of ethyl 3-bromopropanoate are reacted in the presence of sodium hydride, to give ethyl 3-[3-hydroxy-2-(hydroxymethyl)-2-methylpropoxy]propanoate. This compound is subsequently reacted, in the presence of sodium hydride, with one equivalent of tert-butyl 3-bromopropylcarbamate, to give the compound of formula (Y'3a) below:

(Y'3a)



[0124] The compound of formula (Y'3a) above is subsequently deprotected in a trifluoroacetic medium and then reacted with dithioxomethane in the presence of sodium hydroxide, and the carboxylic group is then reprotected with an ethanol/sulfuric acid mixture, to give the compound of formula (Y'3b) below:

(Y'3b)



[0125] The compound of formula (Y'3b) above is subsequently reacted with 1-(3-iodopropyl)-1H-pyrrole-2,5-dione

IN THE CLAIMS

Please amend the claims as follows:

Claims 1-27 (Canceled).

Claim 28 (Previously Presented): A method for detection of an analyte a in a fluid sample, comprising the following steps:

1) saturating a solid support comprising, on at least part of its surface, at least one trifunctional reagent (tripod Y) comprising the following three functional poles:

i) a luminescent group (L),

ii) a molecule (B) selected from the group consisting of the analyte a, an analog of the analyte a or a fragment of the analyte a; and

iii) a function that provides attachment of the trifunctional reagent to the surface of the solid support,

with a receptor for the analyte a, the receptor being labeled with a compound (Q) (receptor-Q) that quenches the luminescence of the group L, so as to form a complex C between the molecule (B) and the receptor-Q;

2) bringing the solid support obtained in step 1) into contact with a fluid sample that may comprise the analyte a to be detected;

3) measuring the intensity of the signal emitted by the group L, which is proportional to the amount of analyte a present in the fluid sample; and

4) regenerating the solid support by bringing the solid support into contact with the receptor-Q.

Claim 29 (Previously Presented): The method as claimed in claim 28, wherein several types of tripods Y that differ from one another through the nature of the molecule (B) that they comprise are attached to distinct and known zones of the solid support.

Claim 30 (Previously Presented): The method as claimed in claim 28, wherein step 3) and step 4) are carried out continuously.

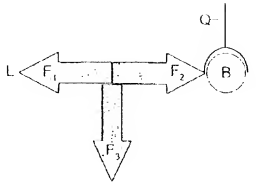
Claim 31 (Previously Presented): The method as claimed in claim 28, wherein the solid support is selected from the group consisting of glasses, plastics, ceramics, metals and metalloids.

Claim 32 (Previously Presented): The method as claimed in claim 28, wherein the solid support is in the form of a tube, a capillary, a plate or a bead.

Claim 33 (Previously Presented): The method as claimed in claim 28, wherein the fluid sample consists of water, a liquid biological medium, or a liquid medium comprising dissolved gaseous molecules or molecules originating from solid samples.

Claim 34 (Previously Presented): The method as claimed in claim 28, wherein the intensity of the signal emitted during step 3) is determined by a luminescence detector.

Claim 35 (Previously Presented): The method as claimed in claim 28, wherein the complex C formed at the end of the saturation in 1) is selected from the group consisting of complexes of formula (I) below:



(I)

wherein:

- the arrows represent the structure of the backbone of the tripod Y, which is a linker arm consisting of a peptide, nucleotide or glucoside chain or of a saturated or unsaturated, linear or branched hydrocarbon-based chain; the chains being optionally substituted, interrupted and/or ended with one or more hetero atoms, such as N, O or S, and/or with one or more amino acids, and comprising three reactive chemical functions F_1, F_2 and F_3;

- L represents a luminescent group covalently bonded to the tripod Y by the reactive chemical function F_1;

- B represents an analyte a, a structural analog of an analyte a or a fragment of an analyte a to which is noncovalently and reversibly attached a receptor specific for the analyte a, the receptor being labeled with a compound Q; the molecule (B) being covalently bonded to the tripod Y by the reactive chemical function F_2;

- Q represents a compound that quenches the luminescence of the group L; and

- F_3 represents a reactive chemical function that can allow the attachment of the tripod Y to the surface of the solid support.

Claim 36 (Previously Presented): The method as claimed in claim 35, wherein the functions F_1, F_2 and F_3, independently of one another, provide:

Claim 40 (Previously Presented): The method as claimed in claim 28, wherein the receptor is selected from the group consisting of antibodies in whole, fragmented or recombinant form, biological receptors, nucleic acids, peptide nucleic acids, lectins, transporter proteins, chelates and synthetic receptors.

Claim 41 (Previously Presented): The method as claimed in claim 28, wherein the receptor exhibits greater affinity for the analyte a than for the molecule (B).

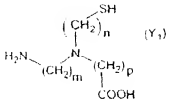
Claim 42 (Previously Presented): The method as claimed in claim 28, wherein the quenching compound (Q) is selected from the group consisting of rhodamine and its derivatives, the fluorescent compounds mentioned in claim 12, and nonfluorescent molecules.

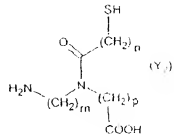
Claim 43 (Previously Presented): The method as claimed claim 35, wherein the complexes of formula (I) are selected wherein:

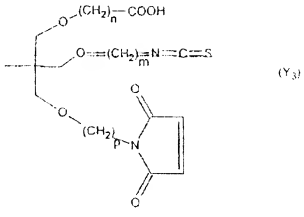
i) (B) is selected from the group consisting of peptides, proteins, oligonucleotides, sugars and peptide nucleic acids,

ii) L is fluorescein, and

iii) the backbone of the tripod Y is selected from the group consisting of the structures Y_1 to Y_3 below:

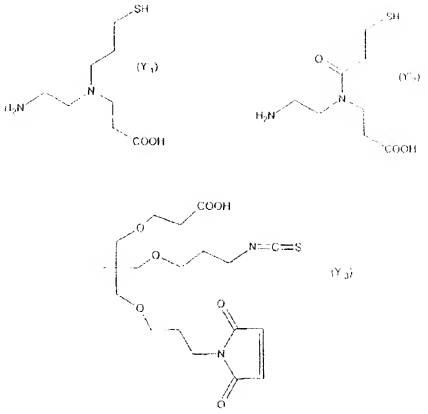




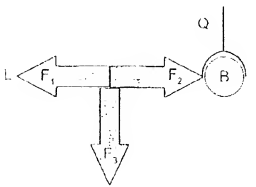


wherein n, m and p, which may be identical or different, are integers between 1 and 20 inclusive.

Claim 44 (Previously Presented): The method as claimed in claim 43, wherein structures Y_1 to Y_3 are selected from the group consisting of compounds of formulae (Y'_1) to (Y'_3) below:



Claim 45 (Previously Presented): A complex C, wherein it corresponds to formula (I) below:



wherein L, B, Q, the arrows and F_1, F_2 and F_3 are as defined in claim 35.

Abstract

The invention concerns a method for heterogeneous phase detection of an analyte, the trifunctional reagent used for implementing said method, its use for detecting an analyte, as well as the device for detecting a corresponding analyte